OLIGONUCLEOTIDES AND THE QUATERNARY STRUCTURE OF GENE-5 PROTEIN FROM FILAMENTOUS BACTERIOPHAGE

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1. Introduction

The gene-5 protein from filamentous bacteriophages fd or M13 provides – in addition to its interesting role in the maturation of those small DNA viruses – an example of a protein, which exhibits strong and cooperative binding to a particular form of DNA. It has been shown, that gene-5 protein shifts the equilibrium from a double-helical form of DNA to a none-helical, apparently disordered, form [1–3]. The extreme cooperativity of binding was deduced from electron microscopy and sedimentation analysis [2]. The stoichiometry of binding was found to be one protein molecule of mol. wt. = 9830 per four nucleotides [1–3].

In an attempt to resolve the individual steps of this cooperative binding process, the interaction of gene-5 protein with oligonucleotides of different length was studied and the quaternary structure of the protein investigated by cross-linking of the protein with dimethyl suberimidate and analysing the reaction product by SDS-gel electrophoresis [4,5].

2. Material and methods

2.1. *Preparation of fd gene-5 protein*

Bacteriophage fd and host strains used for phage growth (*E. coli* K 12 W 945 and K 12 NKL) were obtained from Drs. J. Oey and R. Knippers.

The growth of host cells and the purification of the protein followed essentially the method described by Oey and Knippers [1], except that the final step of purification used a DEAE-cellulose column [2] instead of phosphocellulose. Protein preparations proved to be homogeneous in SDS-gelectrophoresis and show an absorbance ratio 275 nm/255 nm of greater than 2.85. Protein concentration was determined from the absorbance at 275 nm, using an extinction coefficient of 7100 M^{-1} cm^{-1} [1–3].

2.2. *Oligo- and polynucleotides*

The synthesis, purification and characterization of oligo(dG–dC) of different chainlengths and the synthesis of poly (dA–dT) was as described before [6].

2.3. *Optical binding assay*

Binding studies were performed in 20 mM Na-phosphate buffer, pH 7.2, containing 0.1 mM EDTA and 1 mM β-mercaptoethanol, by adding small amounts of protein solution (3.8 mg/ml) to a cuvette containing the nucleic acid. The binding was followed by the change of absorbance at 260 or 280 nm, of the circular dichroism at 230 nm [3], or the decrease in the tyrosine-fluorescence at 310 nm. Another, convenient and sensitive, assay was the decrease of the fluorescence at 585 nm of ethidium bromide (1 μM) bound to polynucleotides upon the addition of gene-5 protein.

2.4. *Cross-linking with suberimidate*

Synthesis of suberimidate and the cross-linking reaction of gene-5 protein was performed essentially as described by Davies and Stark [4]: 10 μl suberimidate (10 mg/ml) was added to a solution containing 45 μl triethanolamine buffer (0.2 M, pH 8.5), 10 μl oligo or polynucleotides (1–4 mM), 5 μl gene-5 protein (3.8 mg/ml) and reacted for 3 hr at room temperature.

2.5. *SDS-gel electrophoresis*

Electrophoresis was performed according to the method of Davies and Stark [4] in 5% or 7.5% poly-

acrylamide. Sodiumdodecylsulfate (SDS) and β-mercaptoethanol were added to the samples at a final concentration of 1%. After heating for 5 min in a boiling-water bath, 30–50 μl of the reaction mixture were applied to the gel. Electrophoresis was carried out at 9 mA per tube for about 2.5 hr using a borate buffer (0.1 M Na-borate, 0.1 M acetic acid, 0.1% SDS). The gels were stained by incubating for 4 hr with 0.07%. Coomassie blue in 45% methanol, 10% acetic acid, 45% water. Gels were scanned at 550 nm with a Beckman Acta III recording spectrophotometer [5].

3. Results

Fig. 1a shows the decrease of the fluorescence intensity of ethidium bromide, bound to poly (dA–dT) · poly (dA–dT), observed with increasing concentrations of gene-5 protein. Similar sigmoidal binding curves were obtained by monitoring the relative change of absorbance at 260 nm due to the 'melting' of poly (dA–dT) by gene-5 protein. Fig. 1 b gives another example, the binding of gene-5 protein to p-(dG–dC)$_3$ · p-(dG–dC)$_3$ as followed by the change of absorbance at 280 nm. Such sigmoidal binding curves were observed even with p-(dG–dC)$_2$, that is with an oligomer of chain length four. In this case one expects a hyperbolic binding curve, since the stoichiometry indicates one protein molecule interacting with one tetranucleotide.

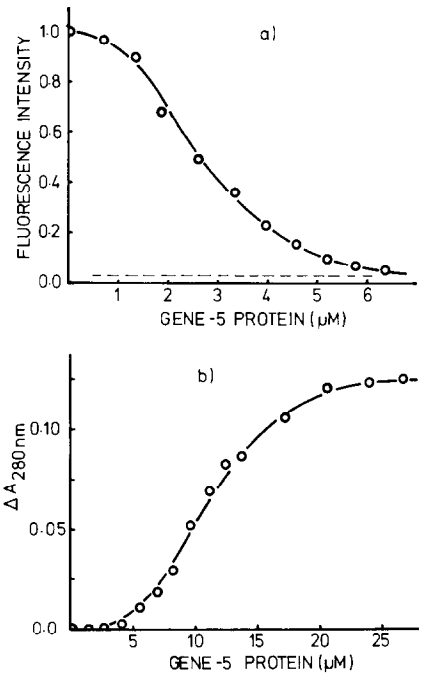

Fig. 1. Titration of nucleic acid with gene-5 protein in 20 mM Na-phosphate, pH 7.2, 1 mM β-mercaptoethanol and 0.1 mM EDTA at 20°C. a) Decrease of the fluorescence of ethidium bromide (1 μM) bound to poly (dA–dT) (11.5 μM) at 585 nm (excitation 520 nm) upon the addition of gene-5 protein; (– – –) fluorescence of free othidium bromide; b) increase of the absorbance of p-d (GCGCGC) at 280 nm as function of the gene-5 protein concentration; corrected for dilution and the contribution by the protein.

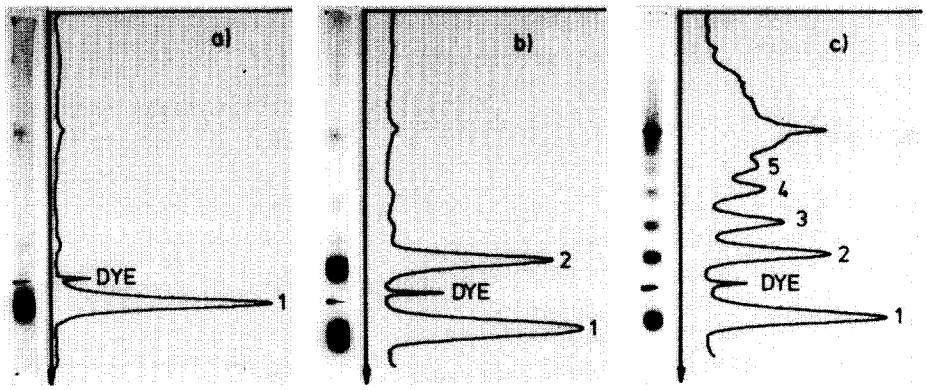

Fig. 2. SDS-gel electrophoresis of gene-5 protein (gels were scanned at 550 nm with arbitrary scale; for details see Materials and methods): a) without cross-linking; b) gene-5 protein (27.5 μM) cross-linked with 1.3 mg/ml suberimidate for 3 hr at room temperature; c) as in b) but in the presence of poly(dA–dT) (150 μM).

The quaternary structure of the gene-5 protein was subsequently studied by chemically cross-linking the molecules in solution with suberimidate and analysing the reaction product by SDS-gel electrophoresis. Representative gel patterns of gene-5 protein with and without cross-linking in the presence and absence of nucleic acids are shown in figs. 2 and 4.

Without cross-linking only one protein band is observed, migrating with an apparent mol. wt. of 10 000 (fig. 2a). In contrast two bands are seen if the protein is allowed to react with suberimidate (fig. 2 b). This shows that the protein in solution exists as a dimer, giving rise to a monomer and a dimer band after cross-linking, in agreement with previous observations [1].

The picture changes considerably, if poly (dA–dT) is present in the solution under otherwise identical conditions (fig. 2 c). A number of additional bands with decreasing mobility are seen, representing cross-linked molecules containing up to about 8 monomers. There appears to be no cross-linking between the high-molecular weight nucleic acid and the protein, which would manifest itself in a band at the origin of the gel. The amount of protein in the higher order bands decreases considerably if 0.3 M NaCl is present, which is in accordance with the reduced binding of gene-5 protein to poly (dA–dT) at high salt concentration.

The additional bands (in fig. 2 c) can be asignated to multiples of the monomeric protein which have been cross-linked in the presence of the nucleic acid.

Fig. 3 shows the plot of the logarithm of the molecular weight of cross-linked molecules against the relative distance R_f of migration into the gel. A nearly linear relationship is found between these two variables. One expects to find this behaviour if band 2 contains two

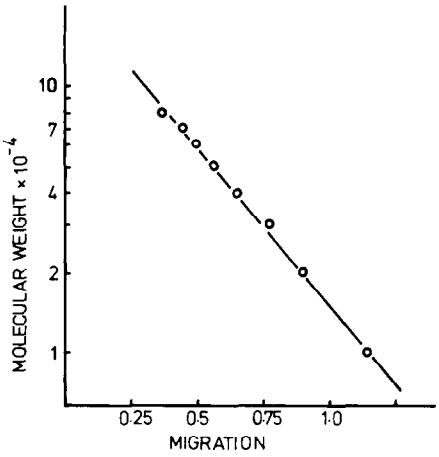

Fig. 3. Logarithm of the molecular weight (band number) of gene-5 protein (cross-linked in the presence of poly (dA-dT) or oligo (dG–dC) as function of the migration relative to bromphenol blue.

molecules, band 3 three protein-molecules, band 4 four molecules etc. cross-linked to each other. The data are consistent with an enhanced association of gene-5 protein on poly (dA–dT). This agrees with the electron

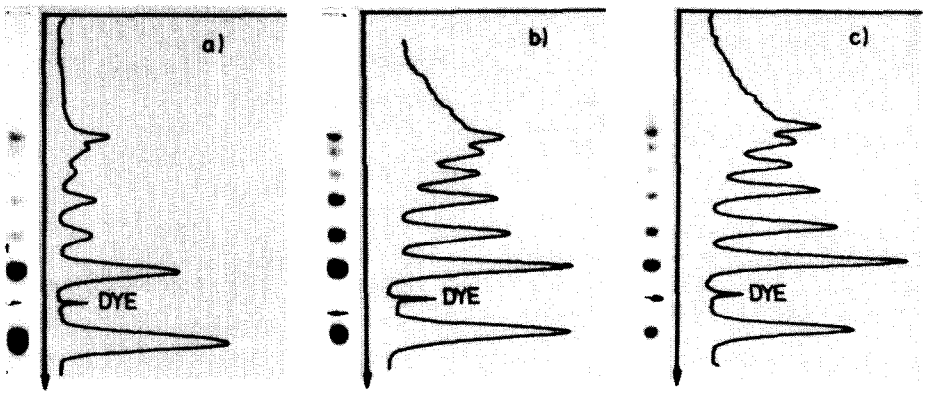

Fig. 4. SDS-gel electrophoresis of gene-5 protein (27.5 μM), cross-linked with 1.3 mg/ml suberimidate in the presence of oligo (dG–dC) of different chainlength. Experimental conditions as in fig. 2: a) p-d (GCGC); b) p-d (GCGCGC); c) p-d (GCGCGCGC).

microscope observations of Alberts, Frey and Delius on fd- or T7-DNA [2].

Fig. 4 shows that this association is not simply due to the presence of a continuous polymeric chain. Oligo (dG–dC) of chainlength four (fig. 4 a), six (fig. 4 b) or eight (fig. 4 c) gives gel patterns very similar to poly(dA–dT), when the protein is cross-linked with suberimidate.

Thus a nucleic acid with a chain length as short as four, that accomodates one protein molecule, gives rise to cross-linked molecules consisting of up to about 6 monomers. Therefore, the enhanced association of gene-5 protein appears to be brought about by the binding to short stretches of nucleic acid chain. Mononucleotides, like dGMP and dCMP, even at a ten times higher concentration do not lead to such an association of gene-5 protein; the gel pattern after cross-linking is essentially the same as in absence of nucleotides.

4. Discussion

The simplest explanation in agreement with the experimental results is that the association of gene-5 protein with itself is greatly enhanced if it binds to a short stretch of nucleic acid, as shown schematically in fig. 5. In contrast, the protein in the absence of a ligand appears to be in the form of 'closed' dimer structure, since no cross-linked molecules higher than dimers have been observed under such conditions. That the association of the gene-5 protein in the presence of nucleic acid is not due to the presence of a continuous chain is shown by the results obtained with oligomers as short as four nucleotides. These short oligomers give rise to gel patterns which are very similar to the ones obtained with polymers. This can be explained by assuming that the binding of short (or long) nucleic acids induces a conformational change of the gene-5 protein, which in turn is responsible for the enhanced association. (Small changes in the circular dichroism spectrum of the protein upon binding to DNA gives some experimental support to this assumption).

The association of the gene-5 protein bound to short pieces is shown schematically in fig. 5 IV. The cross-linking experiments suggest that the protein may act like joints between short pieces of DNA. The observation, that a small protein molecule is able to connect temporarily short pieces of nucleic acids, presumably without a pronounced base specifity [2], may have some bearing on theories concerning the development of different DNA sequences in evolution.

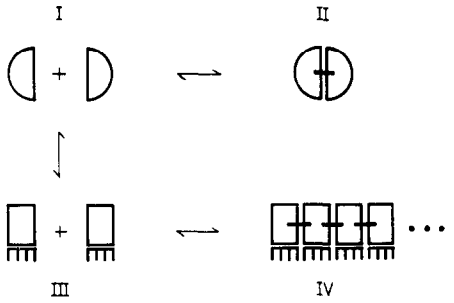

Fig. 5. Schematic presentation of the association of gene-5 protein in the absence (I, II) and presence of oligonucleotides (III, IV) as deduced from cross-linking experiments: (I) monomers of gene-5 in solution (D); (II) association to dimers in the absence of nucleic acids; (III) postulated conformational change of gene-5 protein (□) upon binding of a short oligomeric nucleic acid (┌┬┬┐); (IV) association of gene-5 protein in the presence of tetra-nucleotides. Cross-links between protein molecules are indicated by short bars in (II) and (IV).

Acknowledgements

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References

[1] Oey, J. L. and Knippers, R. (1972) J. Mol. Biol. 68, 125–138.
[2] Alberts, B., Frey, L. and Delius, H. (1972) J. Mol. Biol. 68, 139–152.
[3] Day, L. A. (1973) Biochemistry 12, 5329–5339.
[4] Davies, G. E. and Stark, G. R. (1970) Proc. Natl. Acad. Sci. U.S. 66, 651–656.
[5] Hucho, F. and Changeux, J.-P. (1973) FEBS Letters 38, 11–15.
[6] Pohl, F. M. (1974) Eur. J. Biochem. 42, 495–504.